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PRELIMINARY REPORT ON THE VIRULENCE OF CERTAIN BODY ORGANS IN RINDERPEST [1]

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The following results were obtained while endeavoring to devise a method of securing the aggressins of rinderpest. Since the virus of rinderpest cannot at present be satisfactorily cultivated under artificial conditions, it was decided to try to extract the virus from the tissues of animals suffering from this disease. From the symptoms, lesions, and microscopical findings it is evident that the virus attacks primarily the involuntary muscles and the endothelial lining of the capillary vascular system and the parenchymatous tissue. This is prominently demonstrated in the intestinal tract and in the lymphatic system. Upon microscopical examination of sections of intestine from an animal that has died of rinderpest it will be found that the capillary vascular system in the mucosa is flimsy. The vessel walls are stretched, distorted, and lacking tone and are unable to return to their normal shape. This weakened condition of the vessel walls leads to congestion, diapedesis of red blood cells, and exudation of the blood plasma. As the plasma infiltrates the surrounding tissue, it coagulates, resulting in coagulation necrosis and the formation of fibrinous casts, which are constantly present in the colon of fatal cases. From the result obtained by the intravenous injection of various drugs and disinfectants (4) it is evident that the virus of rinderpest does not have its fountain head of development in the blood stream. The real place where the virus multiplies appears to be

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inside the tissue cells, where the disinfectants cannot penetrate, the virus in the blood stream being merely a surplus that is thrown off from these tissue cells. In following this line of reasoning, it was decided to consider certain tissues, where lesions were more or less pronounced, as cultures, and extracts were made from them.

The tissues used in the following experiments were liver, spleen, lymph glands, heart, intestines, thymus, skeletal muscle, larynx, pharynx, and the back of the tongue from animals that were either bled to death for virulent blood or that had died after a regular course of the disease.

The tissues were taken from the animal as soon after death as possible. The amount of tissue desired was weighed and then ground in a meat grinder that had been previously sterilized in the autoclave to keep external contamination to the minimum. The material thus prepared was placed in a sterilized flask, and twice as much phenol solution (the strength of which will be mentioned in each experiment) was added. Both crude phenol and the pure crystal form were used in these experiments with similar results, that is, 100 grams of liver were ground and placed in a sterile flask, and 200 cubic centimeters of a 0.5 per cent phenol solution were added to it. This material was kept in the refrigerator, which averages between 15° and 16° C., and was daily thoroughly agitated two or three times. In some experiments the material was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature, which averages 26° C. in the morning and 28° C. in the afternoon, some days rising to 30° C. After forty-eight hours of agitation at room temperature the material was placed in the refrigerator for twenty-four hours and then filtered through gauze to separate the coarse material, and the filtrate was replaced in the refrigerator until used.

When the intestines were to be extracted, they were first thoroughly washed free from fæcal matter, then placed in a 0.5 per cent phenol solution for from five to ten minutes, after which they were placed in a large container of boiled water, which was cooled to at least 37° C. The tissue was allowed to soak in this water for a few minutes to dilute the phenol that remained intact. By this method a greater percentage of the bacteria on the surface of the intestinal mucosa was destroyed. Following this treatment the tissue was weighed, passed through the meat grinder, and treated in a manner similar to that of the other tissues.

The animals used in these experiments were all highly sus-

ceptible. They were obtained from localities where, to our knowledge, rinderpest has never been introduced, or from localities where the presence of rinderpest has not been known for many years. These animals were brought to the laboratory and placed under observation for various lengths of time, which will be mentioned in each experiment. During the period of observation in the quarantine shed and throughout the course of the experiments their temperatures were taken twice daily and their general appearance was noted. The animals were kept in an isolation shed while under experimentation and until the first symptoms of disease appeared. Usually one or more susceptible animals were among them to check up any accidental infection that might gain entrance from sources other than by inoculation. As soon as the first symptoms of disease made their appearance, the animal thus affected was immediately transferred to the shed where the sick animals were kept.

The following abbreviation will be used in the experiments:

P. C. W., animals used by the Philip C. Whitaker antirinderpest serum plant.

EXPERIMENT 1

Water extract of liver, spleen, and lymph glands, 3 days old.

Carabao 69.—Known history prior to the experiment: Native Fuga carabao, 3 years old, received at the laboratory and placed in quarantine January 7, 1917. This animal was kept under observation for twelve days before it was used and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 6, 1917, carabao 69 was injected subcutaneously with 100 cubic centimeters of a 3-day-old water extract from the liver, spleen, and lymph glands of carabao 66, which was bled to death on the third day of temperature for virulent blood, to be used in immunization work. Fifty grams of each of these tissues were used, and 300 cubic centimeters of water were added and allowed to extract in the refrigerator. After three days of extraction the material was filtered through gauze, and 100 cubic centimeters of the filtrate were used in this experiment.

February 10, carabao 69 presented an afternoon temperature of 40.5° C.

February 11, forenoon temperature, 40.3° C.; afternoon temperature, 40.8° C.; diarrhœa, not eating.

February 12–13, diarrhœa, not eating.

February 14, died, presenting typical symptoms and lesions of rinderpest.

EXPERIMENT 2

Water extract of liver, spleen, and lymph glands, 3 days old.

Carabao 72.—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for twelve days before it was used and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 6, 1917, carabao 72 was injected subcutaneously with 100 cubic centimeters of the same extract as was used in experiment 1.

February 9, carabao 72 presented an afternoon temperature of 40.5° C.

February 10–12, diarrhœa, not eating.

February 13, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From the results of these two experiments it will be noticed that the watery extract from the organs used was very potent, the incubation period being four and three days, respectively, and the various symptoms leading up to death were prompt in making their appearance after the initial rise in temperature.

EXPERIMENT 3

Phenol (0.5 per cent) extract of liver and lymph glands, 5 days old.

Carabao 75.—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for twenty-two days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 16, 1917, carabao 75 was injected subcutaneously with 200 cubic centimeters of a 4-day-old 0.5 per cent phenol extract from the liver and lymph glands of carabao 73, which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 250 grams; 0.5 per cent phenol, 500 cubic centimeters.
Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator, filtered through gauze on the third day, and returned to the refrigerator. The material had a sweet odor, presenting no evidence of putrefaction.

February 20, carabao 75 presented an afternoon temperature of 39.5° C.

February 21, morning temperature, 39° C.

February 22–23, diarrhœa, not eating.

February 24, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 4

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 5 days old.

Carabao 81.—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for thirty-four days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 28, 1917, carabao 81 was injected subcutaneously with 200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 77, which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 250 grams; 0.5 per cent phenol, 500 cubic centimeters.
Spleen, 175 grams; 0.5 per cent phenol, 350 cubic centimeters.
Lymph glands, 135 grams; 0.5 per cent phenol, 270 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

March 2, carabao 81 presented a forenoon temperature of 39.5° C. and an afternoon temperature of 40° C.

March 4–7, diarrhœa, not eating.

March 8, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 5

Phenol (0.5 per cent) extract of heart, 5 days old.

Carabao 84.—Known history prior to the experiment: Native Fuga carabao, 3 years and 2 months old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for fifty-six days before it was used in this experiment. On March 8, 1917, it was injected with 10 cubic centimeters of culture material in which the virus of rinderpest had been inoculated. The animal presented no ill effects from this injection.

March 22, 1917, carabao 84 was injected subcutaneously with

200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the heart from carabao 85, which was bled to death on the fourth day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Heart, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When this extract was used, it had a sweet odor and presented no signs of putrefaction.

March 28, carabao 84 presented a forenoon temperature of 39.6° C. and an afternoon temperature of 40.7° C.

March 29, diarrhœa.

March 30, diarrhœa, eating little.

March 31, this animal was bled to death for virulent blood, to be used in immunization work. It presented good lesions of rinderpest.

EXPERIMENT 6

Phenol (0.5 per cent) extract of skeletal muscle, 5 days old.

Carabao 88.—Known history prior to the experiment: Jolo carabao, 8 years and 4 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for twenty-six days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

March 29, 1917, carabao 88 was injected subcutaneously with 200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of skeletal muscle from carabao 263 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used for hyperimmunization work in the production of anti-rinderpest serum.

The extract was prepared as follows:

Skeletal muscle, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

Carabao 88 did not developed any ill effects from this injection.

April 15, 1917, which was seventeen days after the muscle-extract injection, this animal was injected subcutaneously with

50 cubic centimeters of virulent blood, to test its susceptibility to rinderpest.

April 19, this animal presented an afternoon temperature of 40.7° C.

April 21–22, diarrhœa, eating little.

April 23–25, diarrhœa, not eating; blood and mucous casts in the fæces.

April 26, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest, which proves that it was highly susceptible to that disease when it was injected with the muscle extract. This result also leaves the impression that the virus of rinderpest is not harbored in the skeletal muscles or that it is easily destroyed in this tissue.

EXPERIMENT 7

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

Carabao 93.—Known history prior to the experiment: Jolo carabao, 8 years and 4 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for fifty-two days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

April 25, 1917, carabao 93 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood to be used for hyperimmunization work in the production of antirinderpest serum.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 29, carabao 93 presented a forenoon temperature of 39.2° C. and an afternoon temperature 40.2° C.

April 30, diarrhœa; afternoon temperature, 40.9° C.

May 1, diarrhœa, eating little.

May 2–3, diarrhœa, not eating.

May 4, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 8

Phenol (0.5 per cent) extract of cæcum and of colon, 5 days old.

Bull 4264.—Known history prior to the experiment: Native Fuga bull, 4 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for three days before it was used; it did not present a high temperature or develop any symptoms of sickness during this time.

April 15, 1917, bull 4264 was injected subcutaneously with 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the cæcum and colon from carabao 251 (P. C. W.), mentioned in experiment 7.

The extract was prepared as follows:

> Cæcum and colon, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 20, bull 4264 presented an afternoon temperature of 40.9° C.

April 21, afternoon temperature, 41.1° C.

April 22–23, diarrhœa, not eating.

April 24, bled to death for virulent blood, to be used in immunization work. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 9

Phenol (0.5 per cent) extract of larynx, pharynx, and base of tongue, 5 days old.

Bull 4265.—Known history prior to the experiment: Native Fuga bull, 4 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for seven days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 20, 1917, bull 4265 was injected subcutaneously with 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the larynx, pharynx, and base of tongue from carabao 240 (P. C. W.), which was bled to death on the third day of temperature for virulent blood to be used in immunization.

The extract was prepared as follows:

Larynx, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.
Pharynx, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.
Base of tongue, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

Bull 4265 did not develop any ill effects from this injection.

May 7, 1917, which was seventeen days after the injection of the above-mentioned extracts, this animal was injected subcutaneously with 50 cubic centimeters of virulent blood.

May 10, bull 4265 presented its first rise in temperature, registering, in the forenoon, 39° C.; in the afternoon, 40° C.

May 11, forenoon temperature, 39.9° C.; bled to death for virulent blood, to be used in immunization work. This animal presented lesions found in the early stages of rinderpest. This proves that bull 4265 was susceptible to the disease when it received the injection of extracts. It also leads to the idea that the virus from these parts is either scarce or is easily destroyed by the above method of handling, as it will be noticed in experiment 10 that other tissues from the same animal were virulent eight days after extraction.

EXPERIMENT 10

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 8 days old.

Bull 4266.—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for ten days before it was used, and it did not present a high temperature or develop any symptoms of sickness during this period.

April 23, 1917, bull 4266 was injected subcutaneously with 50 cubic centimeters of an 8-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 240 (P. C. W.), mentioned in experiment 9.

The extract was prepared as follows:

Liver, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.
Spleen, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.
Lymph glands, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was

then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 26, bull 4266 presented its first rise in temperature, registering, in the forenoon, 38.9° C.; in the afternoon, 40.5° C.

April 28–30 diarrhœa, not eating.

May 1–2, diarrhœa, not eating.

May 3, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 11

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

Three animals were used in this experiment, bulls 4269, 4270, and 4271. All these animals received a similar amount of the same extract on the same day, and all gave similar results. Therefore one only, bull 4269, will be considered.

Bull 4269.—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for twelve days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 25, 1917, bull 4269 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. The liver extract had a slight butyric acid odor when it was injected; the others were sweet.

April 27, bull 4269 presented its first rise in temperature, registering, in the forenoon, 39.1° C.; in the afternoon, 40.3° C.

April 28, forenoon temperature, 40° C.; afternoon temperature, 41.2° C.

April 30, diarrhœa, eating little.

May 1, forenoon temperature, 40.5° C.; bled to death for virulent blood, to be used in immunization work. This ani-

mal presented typical symptoms and lesions of a severe case of rinderpest.

Bulls 4270 and 4271 died of rinderpest during the forenoon of May 4. Both animals presented typical symptoms and lesions of rinderpest.

EXPERIMENT 12

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 16 days old.

Cow 4260.—Known history prior to the experiment: Native Fuga cow, 2 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for twenty-one days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 4, 1917, cow 4260 was injected subcutaneously with 200 cubic centimeters of a 16-day-old 0.5 per cent phenol extract of the liver, spleen, and parotid and lymph glands from carabao 228 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Parotid, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.
Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

May 7, cow 4260 presented its first rise in temperature, registering, in the afternoon, 40.3° C.

May 10, not eating.

May 11–12, diarrhœa, not eating.

May 13, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 13

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 20 days old.

Bull 4272.—Known history prior to the experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation twenty-seven days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 10, 1917, bull 4272 was injected subcutaneously with 200 cubic centimeters of a 20-day-old 0.5 per cent phenol extract of the liver, spleen, and parotid and lymph glands from carabao 228 (P. C. W.), the preparation of which is described in experiment 12. The liver extract had a slight butyric acid odor at the time of injection. The other extracts had a sweet odor and presented no evidence of putrefaction.

May 14, bull 4272 presented its first rise in temperature, registering, in the forenoon, 39.4° C.; in the afternoon, 40.4° C.

May 16–18, diarrhœa, eating little.

May 19–20, diarrhœa, not eating.

May 21, animal died early in the forenoon, presenting typical symptoms and lesions of rinderpest.

EXPERIMENT 14

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

Carabao 92.—Known history prior to the experiment: Native Jolo carabao, 3 years and 6 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for fifty-two days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 25, 1917, carabao 92 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.
Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 28, this animal presented its first rise in temperature, registering, in the forenoon, 39.4° C.; in the afternoon, 39.6° C.

May 1, diarrhœa; forenoon temperature, 39.8° C.; afternoon temperature, 41.1° C.

May 2–3, diarrhœa, not eating.

May 4, diarrhœa, eating little.

May 5, this animal's temperature was normal, and it was well on the road to recovery.

June 1, carabao 92 received 50 cubic centimeters of virulent blood, which had no ill effect upon it, proving that the animal was immune to rinderpest. In addition, this animal was constantly exposed to animals in various stages of the disease.

From this result it appears that the virus in this case had become slightly attenuated by the extraction.

EXPERIMENT 15

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 29 days old.

Carabao 104.—Known history prior to the experiment: Native Jolo carabao, 7 years and 5 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for seventy-one days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 14, 1917, carabao 104 was injected subcutaneously with 200 cubic centimeters of a 29-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 240 (P. C. W.), which was bled to death on the third day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 300 grams; 0.5 per cent phenol, 600 cubic centimeters.
Spleen, 300 grams; 0.5 per cent phenol, 600 cubic centimeters.
Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, the liver had a slight butyric acid odor. The other extracts were sweet, presenting no evidence of putrefaction.

May 24, which was ten days after the injection, carabao 104 presented a forenoon temperature of 39° C.; afternoon, 39° C.

May 25, forenoon temperature, 39.4° C.; afternoon, 41.3° C.

May 26, diarrhœa, eating little; forenoon temperature, 39.9° C.; afternoon, 38.7° C.

May 27, the animal was eating well and its temperature registered normal. This animal rapidly recovered from the slight attack and to date has not presented any signs of rinderpest, although constantly exposed to the disease.

From the results of this experiment it appears that the virus

was markedly attenuated, having just enough vitality to cause a slight onset of the disease, which led to a speedy recovery.

EXPERIMENT 16

Phenol (0.5 per cent) extract of pancreas, 5 days old.

Carabao 107.—Known history prior to the experiment: Native Jolo carabao, 5 years and 6 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation eighty days before it was used and did not present a high temperature or develop any symptoms of sickness during that period.

May 23, 1917, carabao 107 received subcutaneously 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of pancreas from bull 4 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used in serum production.

The extract was prepared as follows:

Pancreas, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

Carabao 107 never developed any ill effects from this injection.

June 13, which was twenty-one days after the pancreas-extract injection, carabao 107 received 50 cubic centimeters of virulent blood.

June 18, carabao 107 presented its first rise in temperature, registering, in the forenoon, 39.8° C.; in the afternoon, 40.6° C.

June 19, diarrhœa.

June 20–21, diarrhœa, not eating.

June 22, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From this result it appears that the pancreas extracted in this manner is not virulent after five days.

EXPERIMENT 17

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 55 days old.

Bull 4285.—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine May 8, 1917. This animal was kept under observation thirty-four days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 12, 1917, bull 4285 received subcutaneously 120 cubic centimeters of a 55-day-old 0.5 per cent phenol extract of liver,

spleen, and parotid and lymph glands from carabao 228 (P. C. W.). This extract was from the same lot used in experiment 12. At this time the liver extract had a slight butyric acid odor.

June 20, bull 4285 presented its first rise in temperature, registering, in the forenoon, 39° C.; in the afternoon, 40.5° C.

June 21, diarrhœa; forenoon temperature, 40° C.; afternoon, 40.6° C.

June 22–24, diarrhœa, not eating.

June 25, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From this result it appears possible to keep the virus in a virulent form for as long a period as fifty-five days in a 0.5 per cent phenol solution.

EXPERIMENT 18

Phenol (1 per cent) extract of lymph glands, 6 days old.

Bull 4296.—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for seventeen days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 18, 1917, bull 4296 received subcutaneously 100 cubic centimeters of a 6-day-old 1 per cent phenol extract of lymph glands from bulls 1036 and 1037 (P. C. W.), which were bled to death on the second day of temperature for virulent blood, to be used in the production of antirinderpest serum.

The extract was prepared as follows:

Lymph glands, 200 grams; 1 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was replaced in the refrigerator.

June 22, bull 4296 presented its first rise in temperature, registering, in the forenoon, 39.2° C.; in the afternoon, 41.° C.

June 24–27, diarrhœa, not eating.

June 28, died during the forenoon. This animal presented typical symptoms and lesions of rinderpest, which proves that a 1 per cent phenol solution will not destroy the virus of rinderpest in the lymph glands over a period of six days, nor does the virus appear to be attenuated by its presence.

EXPERIMENT 19

Phenol (1 per cent) extract of liver, spleen, cæcum, and lymph glands, 17 days old.

Bull 4299.—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June, 1, 1917. This animal was kept under observation for twenty-five days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

The extract to be used was prepared as follows:

Liver, 100 grams; 1 per cent phenol, 200 cubic centimeters.
Spleen, 100 grams; 1 per cent phenol, 200 cubic centimeters.
Cæcum, 100 grams; 1 per cent phenol, 200 cubic centimeters.
Lymph glands, 100 grams; 1 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

June 25, 50 cubic centimeters of each extract were added, making a total of 200 cubic centimeters; to this mixed extract 20 cubic centimeters of a 1–1,000 per cent chlorazene solution were added, and the resulting mixture was returned to the refrigerator.

June 26, 1917, bull 4299 received subcutaneously 100 cubic centimeters of the 17-day-old 1 per cent phenol extract of liver, spleen, lymph glands, and cæcum from bull 1034 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to which the 1–1,000 per cent chlorazene solution had been added on the previous day.

July 1, 1917, bull 4299 presented its first rise in temperature, registering, in the forenoon, 39.9° C.; in the afternoon, 39.8° C.

July 2, forenoon temperature, 40.1° C.; afternoon, 40.2° C.

July 3–5, diarrhœa.

July 6, died in the forenoon, presenting good symptoms and lesions of rinderpest.

This shows that the 1 per cent phenol solution after acting seventeen days and the 1–1,000 per cent chlorazene solution after acting one day upon the mixed tissue extracts did not have any apparent detrimental effect upon the virus of rinderpest.

EXPERIMENT 20

Phenol (1 per cent) extract of lymph glands, 20 days old.

Bull 4302.—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for twenty-eight days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 29, 1917, bull 4302 received subcutaneously 100 cubic centimeters of a 20-day-old 1 per cent phenol extract of lymph glands from bull 1034 (P. C. W.) (see experiment 19).

July 2, this animal presented its first rise in temperature, registering, in the afternoon, 40.2° C.

July 3, forenoon temperature, 39.6° C.; afternoon, 40.2° C.

July 6–7 diarrhœa, not eating; died during the afternoon of July 7. This animal presented typical symptoms and lesions of rinderpest.

EXPERIMENT 21

Phenol (1 per cent) extract of liver, 21 days old.

Bull 4303.—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine June, 1, 1917. This animal was kept under observation twenty-nine days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 30, 1917, bull 4303 received subcutaneously 100 cubic centimeters of a 21-day-old 1 per cent phenol extract of liver from bull 1034 (P. C. W.) (see experiment 19).

This animal ran a rather erratic temperature from July 3 until death.

July 9, diarrhœa; afternoon temperature, 40° C., which was the highest temperature registered during the course of the disease.

July 10, diarrhœa.

July 11, diarrhœa, not eating; afternoon temperature so low that it could not be read.

July 12, found dead in the morning. This animal developed rather atypical symptoms, which do sometimes occur in rinderpest.(3) It presented good lesions of the disease.

EXPERIMENT 22

Phenol (1 per cent) extract of spleen, 21 days old.

Bull 4304.—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for twenty-nine days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 30, 1917, bull 4304 received subcutaneously 100 cubic centimeters of a 21-day-old 1 per cent phenol extract of spleen from bull 1034 (P. C. W.) (see experiment 19).

July 3, this animal presented its initial rise in temperature, registering, in the forenoon, 39.4° C.; in the afternoon, 40.6° C.

July 6–8, diarrhœa, not eating.

July 9, died, presenting good symptoms and lesions of rinderpest.

It will be noticed from the results obtained in experiments 20, 21, and 22 that the 1 per cent phenol had apparently no detrimental effect upon the virus of rinderpest when contained in the lymph glands for twenty days and in the liver and in the spleen for twenty-one days.

EXPERIMENT 23

Phenol (1 per cent) extract of lymph glands, 17 days old.

Bull 4306.—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for thirty-eight days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

July 9, 1917, bull 4306 received subcutaneously 100 cubic centimeters of a 17-day-old 1 per cent phenol extract of lymph glands from carabao 107, which died of rinderpest on the sixth day of temperature (see experiment 16).

The extract was prepared as follows:

Lymph glands, 200 grams; 1 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze and returned to the refrigerator.

This animal ran an atypical course of the disease. On July 10, the afternoon temperature was 39.4° C., which was the highest temperature registered.

July 13–14, diarrhœa, not eating.

July 15, found dead in the morning. This animal presented good physical symptoms and good lesions of rinderpest, but did not develop a high temperature. The disease was very acute, as the animal was dead on the morning of the sixth day after injection.

EXPERIMENT 24

Phenol (2 per cent) extract of spleen, 5 days old.

Bull 4316.—Known history prior to experiment: Native Batanes bull, 6 years old, received at the laboratory and placed in quarantine June 3, 1917. This animal was kept under observation for seventy-two days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

August 15, 1917, bull 4316 received subcutaneously 50 cubic centimeters of a 5-day-old 2 per cent phenol extract of spleen from a bull (P. C. W.) that was bled to death for virulent blood on its second day of temperature. This spleen extract was agitated at room temperature for forty-eight hours.

The extract was prepared as follows:

Spleen, 100 grams; 2 per cent phenol, 200 cubic centimeters.

This was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

August 20, afternoon temperature, 39.8° C.

August 21, forenoon temperature, 38.7° C.; afternoon, 39.8° C.

August 22, forenoon temperature, 39° C.; afternoon, 40.2° C.

August 25–26, eating little.

This animal was given 600 cubic centimeters of antirinderpest serum on August 21, 200 cubic centimeters on August 22, and 100 cubic centimeters on August 25. With the mildness of the attack and the administration of the serum the animal made a speedy recovery.

This animal was constantly exposed to animals sick with rinderpest. On September 13, 1917, it was injected with 2,000 cubic centimeters of a 7-day-old 0.75 per cent phenol extract of liver and lymph glands. It never developed the disease, proving that it had been immunized by its first attack.

September 27, 1917, it was considered hyperimmune and was bled to death for its serum.

This proves that the 2 per cent phenol and the agitation did not destroy the virus of rinderpest, but undoubtedly attenuated it to some extent.

EXPERIMENT 25

Phenol (2 per cent) and glycerin extract of spleen, 5 days old.

Bull 4308.—Known history prior to experiment: Native Fuga bull, 3 years and 3 months old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation seventy-four days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

August 15, 1917, bull 4308 received subcutaneously 50 cubic centimeters of a 5-day-old 2 per cent phenol extract of spleen, to which glycerin was added, from the bull (P. C. W.) mentioned in experiment 24.

The extract was prepared as follows:

Spleen, 100 grams.
Glycerin, 50 cubic centimeters.
Water, 150 cubic centimeters.
Phenol (pure), 4 cubic centimeters.

This was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

Bull 4308 did not develop any symptoms of disease from this injection.

August 29, which was fourteen days after the extract injection, this animal was injected with 50 cubic centimeters of virulent blood.

September 1, this animal presented its first rise in temperature, registering, in the forenoon, 39.5° C.; in the afternoon, 40.1° C.

It ran a rather severe course of the disease, but recovered.

This proves that the virus was destroyed in the spleen extract by the action of the 2 per cent phenol and glycerin.

Several similar experiments were tried, using liver and lymph glands, and it was found that the virus was destroyed in each case where glycerin was added.

EXPERIMENT 26

Phenol (2 per cent) extract of lymph glands, 8 days old.

Bull 4335.—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine August 28, 1917. This animal was used on the day of its arrival; consequently it was not under observation previous to the experiment.

August 28, 1917, bull 4335 received subcutaneously 50 cubic centimeters of an 8-day-old 2 per cent phenol extract of lymph glands from bull 1660 (P. C. W.), which was bled to death on its second day of temperature for virulent blood.

The extract was prepared as follows:

Lymph glands, 100 grams; 2 per cent phenol, 200 cubic centimeters.

This was placed in the shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

This injection had no ill effect upon bull 4335.

September 11, 1917, which was fourteen days after the 2 per cent phenol and lymph gland extract injection, this animal received 50 cubic centimeters of a 5-day-old 0.75 per cent phenol extract of liver and lymph glands from carabao 97, which was bled to death for virulent blood on its fourth day of temperature.

September 13, bull 4335 presented its first rise in temperature, registering, in the forenoon, 39.6° C.; in the afternoon, 39.8° C.

September 14, forenoon temperature, 40.2° C.; afternoon, 40.3° C.

September 15, diarrhœa; bled to death for virulent blood, to be used in immunization work. This animal presented good symptoms and lesions of rinderpest.

This proves that the 2 per cent phenol and the agitation together destroyed the virus of rinderpest in the lymph gland extract after eight days.

Similar results were obtained by treating liver tissue in the same way and for the same length of time.

Heart and intestine extracts were found to lose their virulence in six days when treated in the above-mentioned manner.

DISCUSSION

From the results obtained in the foregoing experiments, it is apparent that the virus of rinderpest held in certain tissues of the body is not injured when extracted with weak solutions of phenol. From many observations that have been made in this laboratory during the past seven years, it has been noticed that the virus of rinderpest is quickly destroyed in decomposing material, either tissue or blood. On the other hand, if virulent blood is drawn under aseptic conditions and placed in sterile containers, the virus will retain its activity for five or six days. If the blood is kept in a clotted form, the virus retains its activity a few days longer. It has been shown in previous work(2) that when the large water leech (*Hirudo boyntoni* Wharton) is allowed to feed upon an animal sick with rinderpest, the virus may remain active for a period of twenty-five days inside the body of the leech. In this case the blood is kept from putrefactive organisms and also in a semianaërobic condition.

By extracting certain organs with weak phenol solutions, the activity of the putrefactive organisms is kept down to the minimum, and thus they have little or no effect upon the virus of rinderpest.

Many times a certain method will work in the hands of the originator, but when placed in other hands the same good results are not obtained. To check this, Drs. Ildefonso Patdu and

Florencio Patenia have made these extracts with no supervision from me, and the results obtained from these extracts were similar to those obtained where I had had full supervision.

To prove that these extracts would work as readily upon animals not used in the laboratory, on three different occasions extracts were given to Dr. D. W. Shaffer and Mr. Thomas L. Bean to be used as virulent material on animals that were used in the production of antirinderpest serum in the Philip C. Whitaker antirinderpest serum laboratory. Doctor Shaffer and Mr. Bean obtained as good results with the extracts as we had in the research laboratory, which proves that these extracts work as readily on animals outside as on those inside of the laboratory.

These extracts have been used in the immunization stations in the provinces, under the supervision of Dr. Stanton Youngberg, chief veterinarian.[2] In preparing extracts for the provinces, we use a 0.75 per cent phenol solution. For ordinary immunization work it is best not to use an extract over 15 days old, as there are other factors that enter in that are apt to delude. We have obtained a considerable number of very gratifying results with old extracts, which will be reported in a subsequent paper. On these occasions the animals presented no reaction to the injection. After a period of two weeks these animals were exposed to rinderpest by various methods, that is, by exposure to sick animals, inoculation with virulent blood, and inoculation with extracts. These animals presented no ill effects from the exposures to which they were subjected, showing that they had been immunized by the primary injection of extract.

From the result obtained by Birch on hog cholera,(1) it is possible that tissue extracts can be used as readily in that disease as in rinderpest, thereby lowering the enormous expense of obtaining virulent material in the production of antihog-cholera serum.

I wish to thank Dr. D. W. Shaffer for the privilege of securing various tissues used in these experiments from animals used by him in obtaining virulent blood in the process of making antirinderpest serum.

CONCLUSIONS

1. From the results obtained in experiments 1 and 2, it is evident that water extracts of the liver, spleen, and lymph glands, 3 days old, are highly infectious to susceptible animals.

2. From the results obtained in experiments 3 and 4, it is

[2] *Phil. Agr. Rev.* (1917), 10.

evident that a 0.5 per cent phenol extract of liver, spleen, and lymph glands, 5 days old, is highly infectious to susceptible animals.

3. From the result obtained in experiment 5, it is evident that a 0.5 per cent phenol extract of heart muscle, 5 days old, is highly infectious to susceptible animals.

4. From the result obtained in experiment 6, it appears that the skeletal muscle is not a suitable tissue for making extracts in the case of rinderpest.

5. From the results obtained in experiments 7, 10, 11, 12, 13, 14, 15, and 17, it is proved that a 0.5 per cent phenol extract of liver, spleen, and lymph glands can hold the virus of rinderpest in a virulent form for periods of time varying from eight to fifty-five days.

6. From the result obtained in experiment 8, it is evident that a 0.5 per cent phenol extract of cæcum and of colon, 5 days old, is highly infectious to susceptible animals.

7. From the result obtained in experiment 9, it is apparent that the larynx, pharynx, and base of tongue are not suitable tissues for making extracts in the case of rinderpest.

8. From the result obtained in experiment 16, it is apparent that the pancreas is not a suitable tissue for making extracts in the case of rinderpest.

9. From the results obtained in experiments 18, 20, and 23, it is evident that a 1 per cent phenol extract of lymph glands, 6, 20, and 17 days old, respectively, is highly infectious to susceptible animals.

10. From the result obtained in experiment 19, it is evident that a 1 per cent phenol extract of liver, spleen, cæcum, and lymph glands, 17 days old, is highly infectious to susceptible animals.

11. From the result obtained in experiment 21, it is evident that a 1 per cent phenol extract of liver, 21 days old, is virulent to susceptible animals.

12. From the result obtained in experiment 22, it is evident that a 1 per cent phenol extract of spleen, 21 days old, is virulent to susceptible animals.

13. From the result obtained in experiment 24, it is evident that a 2 per cent phenol extract of spleen, 5 days old, is infectious to susceptible animals.

14. From the result obtained in experiment 25, it appears that when glycerin is added to a 2 per cent phenol extract that has been agitated for forty-eight hours the virus of rinderpest is readily destroyed.

15. From the result obtained in experiment 26, it appears that in a 2 per cent phenol extract of lymph glands, 8 days old, the virus of rinderpest is destroyed.

16. From certain results mentioned in the discussion, it is advisable to use a 0.75 per cent phenol extract not over 15 days old.

17. From the results obtained in working with rinderpest, it is very plausible that similar or even better results may be obtained with the virus of hog cholera along these lines.

18. The tissues best adapted for this work are the liver, spleen, lymph glands, heart, fourth stomach, cæcum, and colon.

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NOTE ON THE USE OF ORGAN EXTRACTS IN PLACE OF VIRULENT BLOOD IN IMMUNIZATION AND HYPERIMMUNIZATION AGAINST RINDERPEST [1]

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In a paper appearing in this number [2] it will be noted that after an animal has been bled to death for virulent rinderpest blood weak phenol extracts can be made from the liver, spleen, lymphatics, heart, and intestinal tract and that these extracts are as potent as virulent blood.

One of the problems in the production of antirinderpest serum is the reduction in the cost of producing virulent material to be used in hyperimmunization. Various methods have been used with more or less success. Nicolle and Adil-Bey(3) were the first to develop a method by which the virulent material could be increased. When an infected animal presented symptoms of diarrhœa, they introduced into the peritoneal cavity a mixture composed of 3 volumes of normal saline solution and 1 volume of a slightly alkaline solution of Martin's peptone. They introduced 6 liters of this material into yearling cattle (the quantity varying according to the size of the animal), and after three hours the animal was bled to death, the peritoneal cavity was opened, and the fluid was aspirated. After allowing this to coagulate, the clear liquid was drained off and used. The fluid thus obtained gave an increase in virulent material, which was used with success in hyperimmunization.

Ruediger(4) obtained equal results using normal saline solution, which he allowed to remain in the peritoneal cavity from one to two hours before bleeding the animal to death and withdrawing it. The same author(5) also used a 5 per cent sodium citrate solution with equal results.

Holmes(1) diluted the virulent blood with an equal volume of potassium citrate solution and claims the diluted blood gave better results than undiluted defibrinated blood.

Martoglio(2) has developed the latest method, in which he

[1] Published in *Phil. Agr. Rev.* (1917), **10**, 448.

[2] *This Journal, Sec. B* (1918), **13**, 127; also *Phil. Agr. Rev.* (1917), **10**, 410.

claims to increase the virulent material about 70 per cent. His technic is as follows:

> When the infected bovine presents the buccal lesions, usually at the end of the fourth or fifth day of the fever, less commonly at the end of the third, sixth, or seventh, it is immobilized in the stocks and intubed in the jugular and the carotid on the same side. The jugular is put in communication with a capacious glass receptacle placed on a level with the head of the animal and containing saline solution, sterilized and at a temperature of 38° to 39°C., leaving the outlet tube of rubber closed by compression of pincers. * * * The carotid is put in communication with the receptacle which receives the pest blood, and the bleeding begins.
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> When the convulsions preceding the death-struggle begin, the bleeding should stop. The assistant shuts the tube for drawing the blood with a clamp and opens the tube admitting the sodium-chloride solution; immediately the serious symptom-complex changes, the muscular contractions begin to cease, the respiration and pulse that were accelerated become regular and the animal when it has received about as much solution as it has lost blood, enters a period of calm.
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> * * * * * * *
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> We usually inject enough solution to make two and a half times the volume of blood taken, and without ill results. * * * The operation over the animal returns to its shed without assistance. After a lapse of about 5 or 6 hours the animal is bled from the same carotid, this time until it dies.

By this method Martoglio claims to wash out the blood vessels and lymphatic system and obtain a potent virus.

Dr. Stanton Youngberg, chief veterinarian, Bureau of Agriculture, and Dr. D. W. Shaffer, formerly in charge of the Philip C. Whitaker antirinderpest serum laboratory, Manila, have been using a simple method of slightly increasing the production of virulent blood as follows: The injected animal is bled from 2 to 4 liters of blood, depending upon its size, on the second day of temperature; the animal is then allowed to stand overnight, during which time the body has an opportunity to replace the volume of the blood lost; on the following day it is bled to death. In the final bleeding practically as much blood in bulk is obtained as would be procured in a single bleeding, which gives an increase in virulent material corresponding to the amount obtained at the initial bleeding.

TISSUE-EXTRACT METHOD

Any of the above-mentioned methods can be utilized and an enormous increase of virulent material still be obtained by extracting the organs in a weak phenol solution. To illustrate this point, we shall consider the data obtained from an animal of ordinary size, which was bled to death and from whose tissues extracts were made.

Batanes bull 4318, bled to death August 24, 1917.
Amount of virulent blood obtained, 9,000 cubic centimeters.
Weight of organs from which extracts were made:

	Grams.
Liver	1,735
Spleen	350
Lymphatics	260
Fourth stomach	320
Cæcum and colon	2,220
Heart	680
Total	5,655

These organs were passed through a meat grinder and placed in twice their bulk of a 0.75 per cent phenol solution; that is, the cæcum, colon, and fourth stomach were first thoroughly washed free from fæcal matter, then placed in 5 per cent phenol solution for from five to ten minutes, after which they were placed in a large container of boiled water, which was cooled to at least 37° C. These tissues were then allowed to soak in this water for a few minutes to dilute the phenol that remained intact (by this method a greater percentage of the bacteria on the surface of the intestinal mucosa is destroyed). Following this treatment the tissue was weighed, passed through the meat grinder, and treated in a manner similar to that adopted for the other tissues (5,565 grams of tissue from this animal $\times 2 = 11{,}130$ cubic centimeters, the amount of phenol solution that should be added). This material was allowed to extract for three days in the refrigerator, being thoroughly agitated three or four times each day. At the expiration of this period it was filtered through gauze to separate the coarse material, and the filtrate was returned to the refrigerator, ready for use.

From the above-mentioned animal about 11 liters of extract filtrate were obtained, plus the 9 liters of blood, which makes a total of 20 liters of virulent material; under ordinary conditions but 9 liters would have been secured.

If this animal had been handled by the method advanced by Martoglio, a still greater amount of virulent material would have been obtained. Considering that Martoglio obtains a 70 per cent increase in the virulent blood, it would bring the total up to 26,300 cubic centimeters, which would practically triple the output of virulent material from this animal, providing it had been merely bled to death.

Both simultaneous immunization and hyperimmunization have been accomplished with these tissue extracts at the laboratory and in the immunization stations in the provinces.

Doctor Youngberg had the extracts used in simultaneous immunization of carabao at the immunization station at Lubao, Pampanga Province, Doctor Topacio doing the work. These extracts were tried on two different sets of animals.

July 22, 1917, seventeen head of carabao were brought to the station for immunization and were injected with mixed liver, spleen, and lymph gland extract in a 0.5 per cent phenol solution, 5 days old. The doses administered and the final results were as follows: Eight animals received 5 cubic centimeters each of this extract, five animals giving good reactions; four animals received 10 cubic centimeters each of the extract, all of them giving good reactions; two animals received 15 cubic centimeters each of the extract, neither reacting; two animals received 20 cubic centimeters each of the extract, one reacting; one animal received 25 cubic centimeters of the extract without reacting. On August 5, 1917, all the animals that did not react were injected with 25 cubic centimeters of virulent blood, and none of them developed the disease, proving them to be immune. When these animals were injected with the extract, they also received from 250 to 600 cubic centimeters of antirinderpest serum, the amount of serum administered depending upon the size of the animal.

August 17, 1917, fifteen head of carabao, brought into the station for immunization, received 5 cubic centimeters each of an 8-day-old liver extract that had been prepared as follows: Two hundred grams of liver from an animal bled to death on the second day of temperature were passed through a meat grinder, and 400 cubic centimeters of a 0.75 per cent phenol solution were added to it. This material was placed in the refrigerator and thoroughly agitated three or four times a day. After three days' extraction it was filtered through gauze, and the filtrate was returned to the refrigerator, where it was kept until it was 7 days old. The extract was then shipped to the Lubao immunization station for use in the above injections. Of these fifteen animals, seven developed good reactions. Each of these animals received from 400 to 600 cubic centimeters of antirinderpest serum, depending on its size, at the same time the extract was injected. One of the reacting animals that received 400 cubic centimeters of antirinderpest serum succumbed to the disease, while the others made good recoveries. The animals that did not react were injected with 25 cubic centimeters of virulent blood on September 2, 1917, and one developed the disease from the second injection. There is a possibility that this animal did not contract the disease from the extract injec-

tion on account of a slight fault in the technic of administering it. Since the skin of a carabao is thick, it is difficult to use a small injection such as 5 cubic centimeters and be sure one has good penetration of the virus. When working with this type of animal, it is best to give at least 10 cubic centimeters at an injection. If but 5 cubic centimeters of the material is desired, it can be easily diluted to 10 cubic centimeters with 0.85 per cent sodium chloride solution without affecting the activity of the virus, and in this way the necessary amount of material is available.

The animals on which the extract was used were the ordinary type one has to handle in the immunization stations, as they were obtained from localities where rinderpest had been present for a number of years, and many of those brought to the station had passed through the disease by natural contact. Since at present there is no way of identifying the immunity, all animals are subjected to the same treatment. This accounts for the high percentage of nonreactors obtained in this work. From the results obtained by the use of extracts, Doctor Youngberg states that it has the same efficiency as the most potent virulent blood. With a strong strain of virulent blood he usually obtains about 50 per cent reactors on the first injection. With the extract, slightly over 50 per cent of reactions were obtained, or in other words it picked out all the susceptible animals but one. The possible reason for this one not becoming infected from the extract has been mentioned.

In using the extract for hyperimmunization, we have obtained some very satisfactory results, but there have been a few instances where the massive injection of this highly virulent material has resulted in the death of the animal. The possible causes for this will be discussed in connection with these animals.

The first hyperimmunization work with tissue extracts was accomplished by Doctor Patdu, upon Chinese cattle. These animals were imported to the Philippines to be used as work animals. Before they could be shipped to the provinces, they had to be immunized against rinderpest, which was accomplished at the quarantine station.

Fourteen of these Chinese cattle that had passed through an attack of rinderpest during the immunization process were hyperimmunized with fixed tissue extract obtained from three different animals. These extracts were prepared in 0.5 per cent phenol and were 5 days old. Thirteen of the animals received 1,500 cubic centimeters each of this extract, and one received 1,200 cubic centimeters. None of these animals developed any serious

effects from these injections. They had a slight temperature the day following the injections, which soon subsided to normal. After several days these animals were bled approximately 4 liters each. The serum thus obtained has been used in the immunization stations with good results.

In view of the results obtained in hyperimmunizing the Chinese cattle with the extracts, Doctor Youngberg had the extract tried on some animals in the provincial immunization stations. The extract was prepared at the research laboratory, using liver, spleen, lymphatics, and heart in a 0.75 per cent phenol solution. On the fourth day of extraction this material was filtered through gauze and placed in 15-liter demijohns. When it was 6 days old, it was taken by automobile to the San Fernando and the Apalit immunization stations. On the following day Dr. C. H. Leavitt, in charge of the San Fernando immunization station, injected five animals with 2,000 cubic centimeters each of this extract, and Dr. C. H. Decker, in charge of the Apalit immunization station, injected four animals with 2,000 cubic centimeters each.

Doctor Leavitt states that a short time after the injection the animals became stiff, stopped eating, and in the course of a few days they presented the appearance of being paralyzed. All five of the animals injected died. Doctor Decker had a somewhat similar experience and lost two of the four animals.

We then tried the same type of extract at the laboratory on two animals that had recently recovered from rinderpest. These animals were each injected with 2,000 cubic centimeters of a 7-day-old extract in 0.75 per cent phenol solution. In contrast to the above extracts this material had been kept in the refrigerator up to the time of injection. These animals developed a pronounced œdema in the pendent portion of the body, which completely subsided within three days by the aid of slight massaging and the application of warm water each morning. Within four days these animals were in normal condition and never presented any ill effects from the injections up to the time they were bled to death for serum.

From the results thus obtained it is evident that small Fuga and Batanes cattle can withstand 2,000 cubic centimeters of this extract at one injection without any serious disturbance.

A further test was made in trying to locate the cause of the losses incurred by Doctors Leavitt and Decker. In this experiment the extracts were made in a manner similar to that followed for those described above. On the sixth day they were placed in a demijohn and taken to San Fernando, Pampanga, by automobile and returned to the laboratory the following morning.

This was done to expose the extracts to climatic conditions in the same way that the extracts used by Doctors Leavitt and Decker had been exposed.

Two thousand cubic centimeters of this material were injected into a Batanes bull that had recently recovered from rinderpest. This animal developed an œdema similar to that developed by the others. By massaging and giving a warm bath the œdema had practically subsided by the fourth day. On the morning of the fifth day after injection this animal could not rise to its feet, but continued to ruminate and ate a little during the forenoon. In the evening it was practically paralyzed and was found dead the next morning. From the results obtained in this experiment and those obtained by Doctors Leavitt and Decker it is evident that by exposing these extracts to the climatic conditions existing in the Philippines for a period of twenty-four hours they pass through certain chemical changes, which are very detrimental to animals receiving the extracts in large quantities. The exact changes have not been determined, but they appear to be protein decomposition or botulism toxin.

We are doing further work on trying to eliminate the small particles of tissue that pass through the gauze, by first filtering the extract through gauze, then through a layer of cotton (method similar to that used in filtering agar), and finally passing it through filter paper. By this method we obtain a slightly turbid, dark amber-colored liquid. A small Fuga bull that recently recovered from rinderpest has been injected with 2,000 cubic centimeters of this material, which was kept in the refrigerator up to the time of injection. This material caused a much milder œdema, which practically subsided in two days, and the animal suffered no apparent ill effects. There was a slight elevation of temperature for two days, but the animal continued to eat well and looked bright.

CONCLUSIONS

1. Considering the results thus far obtained, it is evident that tissue extracts from animals suffering with rinderpest are just as potent as virulent blood when used in simultaneous immunization work.

2. Any of the methods advocated for increasing the production of virulent material can be utilized, after which the organs can be extracted, thereby obtaining a much greater increase in quantity.

3. By using Martoglio's method and extracting the organs, the output of virulent material is practically tripled.

4. If the extracts are kept at a temperature of approximately 15° C., they can be used with safety in 2,000 cubic centimeter doses for hyperimmunization.

5. Considering our results up to date, the extracts should not be given in massive injections if they have been exposed for a period of eighteen hours to the climatic conditions found in the tropics.

6. These extracts can be produced so easily that this method can be used in any immunization station.

7. Considering the similarity of hog cholera to rinderpest, this method should be as applicable in that disease as it is in rinderpest, thereby reducing the enormous cost of the virus.

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COMPARATIVE STUDY ON NATURAL HEMOLYSINS IN INACTIVATED HUMAN AND MONKEYS' SERUM [1]

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The absence in human serum of natural hemolytic amboceptor toward the red corpuscles of the monkey has been mentioned in a previous communication.[2]

This study suggested the present experiments, in which twenty-three additional samples of human sera were tested with regard to their content of antimonkey natural hemolytic amboceptor. All these samples, showing a lack of antimonkey hemolytic amboceptor, behaved in the same manner as the forty samples of human sera previously tested.[3]

Kolmer and Casselmann [4] have recently studied the hemolysins in inactivated human serum and found that the said serum contains natural hemolysins toward red cells of the following animals: Sheep, dog, calf, goat, pig, rat, chicken, horse, rabbit, and guinea pig.

On account of our suggestion to substitute, with advantage, monkeys' corpuscles for human red cells in performing the complement fixation test for diagnosis of syphilis, it seemed of interest to compare human serum with that of the monkey with regard to hemolytic amboceptor toward red cells of some of the animals used by the above-mentioned authors.

In Table I there are recorded the results obtained by testing twenty-three inactivated human sera and one monkey's serum for hemolysins against human red corpuscles and those of the monkey, the sheep, the horse, the cow, the goat, the carabao, and the guinea pig.

Technic.—The sera were heated between 55° and 56° C. for thirty minutes. The amount used in the test was 0.2 cubic

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[2] *This Journal, Sec. B* (1917), 12, 249.

[3] Loc. cit.

[4] *Journ. Inf. Dis.* (1915), 16, 441.

centimeter. To each tube were added 1.5 cubic centimeters of physiologic salt solution (0.9 per cent) and 0.5 cubic centimeter of a 4 per cent red-cell suspension. The tubes were allowed to stand at room temperature for one hour, and during this time each tube was frequently shaken. After one hour of exposure to room temperature, 0.5 cubic centimeter of ten times diluted complement was added, using the pooled sera of three guinea pigs. The tubes were placed in the incubator for another hour, and then the results were recorded.

It is evident from the results of these tests (Table I) that none of the human sera showed the presence of natural hemolysins against human, monkeys', horses', and guinea pigs' red corpuscles, but the majority of the same sera contained hemolysins for sheep's and goats' corpuscles and in a slight degree for cows', carabaos', and rabbits' red cells.

The one monkey's serum showed lack of hemolysins against human, monkeys', horses', cows', carabaos', rabbits', and guinea pigs' corpuscles, and like the human sera this one serum contained a great amount of natural hemolysins against sheep's and goats' corpuscles.

The human, as well as the one monkey's serum, showed a strong agglutination of carabaos' and rabbits' red cells. As our first experiment was carried out with only one sample of one monkey's serum, we tested in a second experiment the sera of five different monkeys. These sera gave identical results with the one sample previously tested, as is shown in Table II.

Having established the fact that human and monkeys' sera behave in a similar way with regard to hemolysins toward red cells of various animals, we proceeded in the next experiment to test the sera of these animals, including that of man and of monkey, with regard to the presence or absence of natural hemolytic amboceptor toward the red cells of each of the animals, including man. The results of these tests are evident from Table III. The technic applied in this experiment was the same as in previous tests, except the amount of serum used, which was decreased to 0.1 cubic centimeter.

It is evident from the results of the tests given in Table III that monkeys' serum behaves in the same way as human serum does with regard to natural amboceptor toward the various red corpuscles used in the test. Furthermore human red cells and those of a monkey behave in practically the same way when exposed to the action of inactivated sera of various animals and guinea pigs' complement.

One striking thing, which may be of interest, is the finding that rabbits' serum contains no hemolytic amboceptor for human corpuscles, while it has a large amount of natural hemolytic amboceptor for monkeys' red corpuscles. Monkeys' red cells behave in that respect in a similar way as those of the sheep, the horse, and the goat. This finding probably explains the fact that artificial antimonkey amboceptor of as high titer as that of the sheep, the goat, and the horse can be produced.

This question is being studied in further experiments now under way.

The object of these experiments is to test more than one rabbit's serum and to study the influence of immunization on the amount of natural hemolytic amboceptor.

CONCLUSIONS

1. Inactivated human sera contain no natural amboceptor for monkeys' red corpuscles, but a great percentage of human sera contains a large amount of hemolytic amboceptor for sheep's and goats' corpuscles.

2. Inactivated monkeys' serum contains no natural amboceptor for human red corpuscles, but contains a large amount of natural hemolytic amboceptor for sheep's and goats' corpuscles.

3. Natural hemolytic amboceptor of human and monkeys' sera are almost identical, not having the same relation in this respect with the sera of the sheep, the horse, the cow, the goat, the rabbit, and guinea pig.

4. The serum of the rabbit (one animal) shows hemolysins for the corpuscles of the sheep, the horse, the monkey, and the goat.

TABLE I.—*Showing comparative tests of human sera and one monkey's serum.*

[Inactivated serum, 0.2 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter; —, no hemolysis; +, trace of hemolysis; +2, slight hemolysis; +3, moderate hemolysis; +4, strong hemolysis; +5, almost complete hemolysis; +6, complete hemolysis; a, agglutination.]

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	Monkey. 1	Human— 1 M. A.	2 B. R.	3 G. N.	4 G.	5 B. L.	6 A. K.	7 D.	8 A. A.	9 M. C.	10 T. O.	11 F. B.
Man	—	—	—	—	—	—	—	—	—	—	—	—
Monkey	—	—	—a	—a	—	—a	—	—	—a	—a	—	—
Sheep	+6	+5	+3	—a	—	—	+5	—	+6	+6	+3	+3
Horse	—	—	—a	—a	—	—	—	—	—a	—	—	—
Cow	—	—	—	—	—	—	—	—	+	—	+	+3
Carabao	—a	—a	—a	—a	—a	—a	—a	—a	+a	+a	+a	+a
Goat	+5	+6	+3	—	—	—	+3	—	+5	+6	+6	+6
Rabbit	—a	—a	—a	—a	—a	—a	—a	—a	—a	+a	—a	—a
Guinea pig	—	—	—	—a	—	—	—	—	—a	—	—a	—

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	Human— 12 I. S.	13 F. F.	14 V. G.	15 S. S.	16 L. B.	17 F. C.	18 A. R.	19 F. F. C.	20 Y. B.	21 E. B.	22 U. S.	23 C.
Man	—	—	—	—	—	—	—	—	—	—	—	—
Monkey	—	—	—	—	—	—	—	—	—	—	—	—
Sheep	+3	+6	+6	+6	+3	+3	+6	+6	+6	+5	+6	—
Horse	—	—a	—	—	—	—	—	—	—	—	—	—
Cow	—	—	—	+	+	—	—	—	—	+3	—	—
Carabao	—a	—a	—a	+2a	—a	—a	+2a	—a	+a	+a	+a	—a
Goat	+2	+6	+6	+6	+3	+3	+6	+6	+5	+3	+6	—
Rabbit	—a	—a	—a	+2a	—a	—a	—a	—a	—a	+2a	—a	—a
Guinea pig	—a	—	—	—a	—	—	—	—	—	—a	—	—

TABLE II.—*Showing tests of five different samples of monkeys' serum.*[a]

[Inactivated monkeys' serum, 0.2 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter.]

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.
Man	—	—	—	—	—
Monkey	—	—	—	—	—
Sheep	+5	+4	+4	+5	+5
Horse	—	—	—	—	—
Cow	—	—	—	—	—
Goat	+4	+2	+2	+5	+5
Rabbit	—a	—a	—a	—a	—a
Guinea pig	—	—	—	—	—

[a] See Table I for abbreviations and signs.

TABLE III.—*Showing cross tests of sera and red cells of various animals, including man.*[a]

[Inactivated serum, 0.1 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter.]

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	Human.	Monkey.	Sheep.	Horse.	Cow.	Goat.	Rabbit.	Guinea pig.
Man	—	—	—	—	—	—a	—	—
Monkey	—	—	—	—	—	—	+4	—
Sheep	+	+6	—	—	+3	—	+6	—
Horse	—	—	+	—	+2a	—	+5	—
Cow	—	+	—	—	—	—	—	+
Goat	+	+5	—	—	+5	—	+4	—
Rabbit	—a	+2a	—	—	—	—a	—	—
Guinea pig	—	—	—	—	—	—	—	—

[a] See Table I for abbreviations and signs.